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EXAMINER	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/536,495	BRO ET AL.	
	Examiner	Art Unit	
	MARIA LEAVITT	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12 February 2009*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,11-18* is/are pending in the application.

4a) Of the above claim(s) *14-16* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1, 11-13,17 and 18* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Detailed Action

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 01-12-2009 has been entered.
2. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.
3. Claims 1 and 11-18 are pending. Claims 1, 11-14 have been amended, claims 2-9 have been cancelled and claims 17 and 18 have been added by Applicant's amendment filed on 01-12-2009. Applicants' election of the following species with traverse was previously acknowledged: *Saccharomyces cerevisiae*, ethanol, and glycerol as the undesired product. Claims 14-16 were previously withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on 12-13-2007.
4. Accordingly, claims 1, 11-13, 17 and 18 are currently under examination to which the following grounds of rejection are applicable.

Response to arguments

Response to Applicants' arguments in relation to restriction requirements.

At page 4 of the Remarks filed on 01-12-2009, Applicants essentially argue that the claims of withdrawn Group II, claims 14-16, are method claims dependent on claim 1. Hence, if claim 1 is deemed allowable, claims 14-16 will be rejoined pursuant to MPEP 821.04.

The examiner agrees with Applicant that where applicant elects claims directed to the product, and a product claim is subsequently found allowable, withdrawn process claims that depend from or otherwise include all the limitations of the allowable product claim will be rejoined in accordance with the provisions of MPEP 821.04. Process claims that depend from or otherwise include all the limitations of the patentable product will be entered as a matter of right if the amendment is presented prior to final rejection or allowance, whichever is earlier. Amendments submitted after final rejection are governed by 37 CFR 1.1 16; amendments submitted after allowance are governed by 37 CFR 1.312.

It is also noted that because the genus claim is not allowable as originally claimed, no other species will be rejoined for search and examination.

Withdrawn objections/ rejections in response to Applicants' arguments or amendments:

Claim Rejections - 35 USC § 112 (second paragraph)

In view of Applicants' amendment of claim 1, rejection of claims 1, 6, 7 and 13 under 35 U.S.C. 112, second paragraph, as being indefinite in that it fails to point out what is included or excluded by the claim language has been withdrawn.

In view of the withdrawn objection, applicant's arguments are rendered moot.

Rejections maintained in response to Applicants' arguments or amendments

Claim Rejections - 35 USC § 103

The present invention is drawn to a metabolically engineered *S. cerevisiae* expressing a heterologous recombinant *Streptococcus mutants* GapN gene on a multicopy plasmid which encodes for the **non-phosphorylating $NADP^+$- dependent glyceraldehyed-3-phosphate dehydrogenase (GAPN or GAPDHN)** (p.11 lines 29-32). GAPN catalyses the irreversible oxidation of glyceraldehyde-3-phosphate and $NADP^+$ into 3-phosphoglycerate and NADPH. The reaction catalysed by GAPN yields one NADPH instead of one NADH and one ATP when comparing with the total reaction catalysed by **NAD^+-dependent glyceraldehyde-3-phosphate dehydrogenase (GAPDH)** and subsequent reaction by phosphoglycerate kinase (**PGK or pgk**) (p. 10, lines 14-32). Additionally, the specification discloses that a major problem in connection with ethanol production by anaerobic fermentation of S. *cerevisiae* is a substantial formation of glycerol as a by-product with loss of carbon in the bio-product (e.g., ethanol, amino acid, antibiotics) (p. 2, lines 14-16) . Moreover, the specification discloses, "anaerobic growth of S. cerevisiae on a fermentable sugar, surplus amounts of NADH are formed which cannot by used in generation of ATP, and this results in the formation of by-products, primarily glycerol" (p. 4, lines 14-19). Under anaerobic conditions cytosolic NADH formed from biomass formation can only be reconverted to NAD+ via glycerol formation (p. 2, lines 19-24). Thus, "Glycerol is formed by S. cerevisiae during anaerobic growth to maintain the cytosolic redox balance. Under anaerobic conditions NADH, produced as a result of production of biomass and organic acids, can only be oxidised to NAD+ by formation of glycerol, since respiration is not possible and the formation of ethanol is a redox-neutral process. The formation of glycerol is therefore a redox

problem, so by introducing gapN into *S. cerevisiae"* the following metabolic modification are observed: the production of glycerol is reduced by one molecule for each molecule of glyceraldehyde-3-phosphate that is converted via GAPN and the flux redirected to ethanol and/or biomass thereby increasing the ethanol yield (p. 12, lines 19-32).

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1, 11-13 remain rejected and claims 17 and 18 are newly rejected under 435 U.S.C. 103(a) as being unpatentable over of Nissen et al., (Metabolic Engineering, 2000, 2: pages 69-77) in view of Valverde et al., (FEBS, 1999, 21898, pages 153-158).

Nissen et al., teaches a metabolically engineered *S.* cerevisiae for enhanced production of ethanol wherein reduced formation of surplus NADH and an increased consumption of ATP in

biosynthesis results in decreased glycerol yield. Specifically, Nissen et al., describes a mutant *S. cerevisiae* wherein the normal NADPH-consuming synthesis of glutamate from ammonium and 2-oxoglutarate was substituted for a new pathway in which ATP and NADH were consumed. Nissen created a metabolically *S.* cerevisiae mutant in which *GLN1*, encoding glutamine synthetase, and *GLT1*, encoding glutamate synthase, were overexpressed, and *GDH1*, encoding the NADPH-dependent glutamate dehydrogenase, was deleted in an attempt to increase ethanol formation by reduction of glycerol synthesis (p. 70, col. 2, paragraph 4). The mutant *S.* cerevisiae reoxidized NADH to NAD+ in the synthesis of glutamate from ammonium and 2-oxoglutarate (e.g., ammonium is a source of nitrogen in industrial fermentation of *S. cerevisiae*) resulting in a reduced surplus formation of NADH, increased consumption of ATP and lower glycerol yield. Nissen et al., teaches that excess NADH produced during biomass and organic acids is balanced by glycerol in anaerobic fermentations to avoid a serious imbalance in the NAD+/NADH ratio (p. 69, col. 2, last paragraph). Furthermore, Nissen et al., teaches that if Glt1p and Gln1p catalyzed the reaction instead of Gdh1p, 1 mol NADH and ATP each would be consumed per mole of glutamate synthesized instead of 1 mol of NADPH leading to decrease formation of glycerol and an increase in ethanol formation (page 70, col. 2, last paragraph) . Also note that the metabolically engineered *S. cerevisiae* implicitly has a first metabolic pathway in which a first reaction metabolite, e.g., Glyceraldehyde 3-phosphate, is transformed into a second metabolite, e.g., 1,3-Bisphosphoglycerate (1,3-BPGA), in a reaction in which a NAD+ is a cofactor for a first enzyme, e.g., Glyceraldehyde 3-phosphate dehydrogenase, and in which the second metabolite is transformed by a kinase into 3-Phosphoglycerate. Additionally, Nissen et al., states that a number of byproducts are formed during an anaerobic fermentation of *S. cerevisiae* of

which Glycerol is the most important, consuming up to 4% of the carbon source in industrial fermentation. Thus clearly Nissen et al., discloses metabolically engineered *S.* cerevisiae wherein the flux of carbon from glycerol was redirected to ethanol by substitution of NADP-oxidizing reactions in the yeast with NADH-oxidizing reactions. (Current claims 1, 11-13, 17 and 18, in part).

Nissen et al., does not specifically teach a metabolic engineering *S. cerevisiae* in which the flux of carbon from glycerol was redirected to increase ethanol production by reduced formation of surplus of NADH and ATP resulting from the enzymatic activity a non-phosphorylating NADP-dependent G3P dehydrogenase.

However, at the time the invention was made, Valverde et al., discloses a metabolic engineering glycolytic pathway with no energy yield (e.g, no ATP production) wherein a functional heterologous **non-phosphorylating NADP-dependent glyceraldehyed-3-phosphate dehydrogenase (GAPDHN)** was expressed in *E. coli* resulting in net oxidation of Glyceraldehyde 3-phosphate to 3-phosphoglycerate (3PGA) and reduction of $NADP^+$ to NADPH. Moreover, Valverde et al., discloses that GAPDHN bypasses the first substrate level phosphorylation step of the glycolysis, i.e., conversion of Glyceraldehyde 3-phosphate into 1,3-Bisphosphoglycerate (p. 152, col. 2, paragraph 2). Thus, in contrast to the cytosolic NAD-dependent phosphorylating D-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) which is responsible for the first substrate level phosphorylation step of the glycolysis, producing 1,3-Bisphosphoglycerate, the substrate of 3-Phosphoglycerate kinase (PKG or pkg) which enzymatic activity produces 3-PGA and ATP, NADP+ dependent GAPDHN exhibits a metabolic engineering catabolic glycolytic pathway with no net substrate level phosphorylation and

NAPDH (p. 153, col. 2, paragraph 3; page 157, Fig. 4). Moreover, Valverde et al., teaches phosphorylating and non-phosphorylating branches of the glycolysis wherein GAPDHN complements the W3CG strain defective in GAPDH (see Fig. 4, dashed arrows in the phosphorylating branch box). In addition, Valverde et al., teaches that the GAPDHN is able to function in its natural environments which can compete with advantage over the cytosolic NAD-dependent GAPDH due to its lower Km values for G3P and the pyridine nucleotide (p. 157, col. 1, last paragraph) clearly disclosing a new metabolic engineered redox pathway were cells are able to increase the formation of NADPH at the cost of NADH formation because both GAPDHN and GAPDH compete for the same G3P substrate. Indeed, Valverde et al., discloses that GAPDHN has been found in some prokaryotic microorganisms including Streptococcus strains that lack the NADPH-generating enzymes of the oxidative pentose pathway and these strains simultaneously contain the NAD-depending phosphorylating GAPDH either to generate NADPH or ATP. Thus Valverde does not specifically disclose a recombinant *E. coli* wherein G3P is oxidized to 3-PGA with an inactive kinase, e.g., phosphoglycerate kinase (pgk), Valverde clearly discloses a modified non-phosphorylating, irreversible bypass for G3P → 3-PGA catalyzed by GAPDHN wherein endogenous GAPDH and pgk are functional or, alternatively, GAPDH may be deleted. Thus if GAPDHN would functionally oxidized G3P → 3-PGA in a recombinant *E. coli* with a mutated inactive GAPDH, it should reasonably be expected that GAPDHN would functionally oxidized G3P → 3-PGA in a recombinant *E. coli* with a mutated inactive pgk because pgk is a downstream kinase in the conversion of G3P → 3-PGA, i.e., 1,3-BPGA → 3-PGA. Thus, the catabolic yield of the non-phosphorylating glycolytic pathway catalyzed by **GAPDHN** is one NADPH as compared to one NADH and one ATP produced by

the reaction catalyzed in the successive coupled reactions by the enzymatic activities of GAPDH and pgk, clearly disclosing a new metabolic engineered redox pathway were cells are able to increase the formation of NADPH at the cost of NADH formation because both **GAPDHN** and **GAPDH** compete for the same G3P substrate. Moreover, Valverde et al., concludes "this work also illustrates the possible application of this strategy in biotechnology of fermentative processes".

Therefore, in view of the benefits of metabolic engineered *S. cerevisiae* wherein reduced formation of surplus NADH and an increased consumption of ATP in biosynthesis results in decreased glycerol yield and enhanced ethanol production as taught by Nissen, in part, contributing to reduce net surplus of intracellular NADH (e.g., NADH is reoxidized to NAD+) , it would have been *prima facie* obvious for one of ordinary skill in the art to genetically manipulate other redox metabolic pathways and enzymes leading to overall increase of production in ethanol and to decrease glycerol production, thus avoiding excess NADH produced during biomass formation in fermentation that is counterbalanced by glycerol, new redox pathways that could reduce formation of surplus NADH and reduced production of ATP using the yeast cell by introducing a recombinant **GAPDHN** which will lead to no net substrate level phosphorylation (e.g. reduced overall ATP production) and irreversible oxidation of G3P $\rightarrow$ 3-PGA with production of NADPH at the cost of NADH. This would clearly reduce surplus of NADH produced by GAPDH as both enzymes **GAPDHN and** GAPDH compete simultaneously for the same substrate as disclosed by Valverde. The manipulation of previously identified enzymes and diversion of metabolic flux requiring reducing equivalents, NADPH or NADH in fermenting reactions for increased ethanol production and reduced glycerol formation is within

the ordinary level of skill in the art. One of ordinary skill in the art would have been motivated to clone and express a recombinant GAPDHN in *S. cerevisiae* in an attempt to provide improved production of ethanol by reducing formation of surplus NADH by bypassing the ATP –reaction and by using NADP as a cofactor rather than NAD+, as a person with ordinary skill in the art has good reason to pursue the known options within his technical grasp. In turn, because the metabolic engineered *S. cerevisiae* expressing a recombinant **GAPDHN,** with reduced surplus amount of NADH and no ATP production, showed reduction of glycerol production and increase of ethanol as predicted in the prior art, it would have been obvious to combined the disclosures of Nissen and Valverde to generate the claimed metabolic engineered *S. cerevisiae.*

Response to Applicants' Arguments as they apply to rejection of claims 1, 11-13, 17 and 18 under 35 USC § 103

At pages 7-9 of Remarks, Applicants essentially argue that Nissen does not disclose reducing formation of NADH and ATP by the enzymatic activity of a non-phosphorylating dehydrogenase (e.g., GAPN aka GAPDNH). Indeed, Applicants contend that Nissen when speaking of reduced formation of surplus NADH refers to provision of a pathway for consuming NADH. Thus applicants argue that the Examiner statement at page 12, lines 4 of the action of 03-12-2008, "that because Nissen et al taught that reducing formation of NADH and increasing consumption of ATP resulted in decreased glycerol formation in yeast, it would have been obvious from Valverde et al that this effect could be obtained also by expressing GAPN in yeast to produce 3-phosphoglycerate with production of NADPH rather than NADH + ATP", is miss-stated. Moreover, Applicants essentially formulate their arguments on whether the mechanisms described by Nissen and Valverde are different and distinct mechanisms for reducing surplus of

NADH, in part, because Nissen does not teach a reduction in the formation of NADH but rather a reduction of surplus NADH formation upstream of glycerol production. Furthermore, at page 9 of remarks, Applicants compares on a table the metabolic pathways resulting in reduced formation of surplus NADH and an increased consumption of ATP in biosynthesis upstream glycerol production in Nissen and Valverde. Thus at pages 8-18 of Remarks, Applicants essentially assert that 1) that the metabolically engineered *E. Coli* in Valverde has decreased growth rate as compared to the wild type under anaerobic conditions whereas the metabolic engineered *S. cerevisiae* of Nissen for enhanced production of ethanol grows under anaerobic conditions so the skilled artisan would no perceived Valverde et al as offering a teaching likely to be useful (Remarks at page 10, last paragraph; page 11, first paragraph; page 16, second paragraph, for example), 2) that Valverde does not disclose a strain of *E. Coli* engineered to contain GapN without disablement of production of GAPDH. Indeed, Applicants allege that Figure 2B shows unambiguously clear specific antibodies directed against each of the GAPH used, evidencing that in W3cG/pFVNP1 only GAPDHN from *P. salivum* is detected and in the other three different GAPH tested, only GAPH is seen and not any GAPN. Thus Valverde does not teach a W3CG mutant with both GAPDH and GAPN. Applicants assert that the instant invention requires that the yeast should have an intact GAPDH which produces NADH (at page 11, paragraphs 3-4; pages 12 and 13, for example), 3) Valverde et al., has nothing to say about the balance of production of any product, let alone of glycerol/ethanol. Valverde contains no mention of "surplus NADH" nor discusses any problem with the surplus of NADH or any desire to reduce NADH production. Valverde et al., is merely an academic study investigating what glycolytic pathways may be operative in photosynthetic eukaryotic by investigating one

particular enzyme's activity in *E. Coli* as a test system (at page 13, paragraphs 6-7: page 14 paragraphs 2-3, for example), 4) the teachings of Nissen related to anaerobic fermentation in Yeast and the teachings of Valverde confined to *E. Coli* on the ability to metabolize sugar lost via deletion of GAPDH by introducing GAPN under aerobic conditions are very different fields as they are concerned with the metabolism of yeast and bacteria, respectively. Applicants' arguments filed 01-12-2009 have been fully considered but they are not persuasive.

Regarding 1), the fact that Valverde discloses a metabolically engineered *E. Coli* with deleted GAPDH and expressing a functional GAPDHN with reduced growth under anaerobiosis with specific glucose supplemented substrate conditions, does not preclude GAPDHN from being functional in *E. Coli* growing in anaerobic conditions under different metabolic requirements wherein glycolysis may not be the only energy supplying route for the enterobacterium. Indeed, Valverde illustrates in Fig. 3 cited by Applicants reduced but not abrogated growth of *E. Coli* under anaerobic conditions when culture medium is supplemented with succinate plus glycerol rather than glucose, clearly indicating that expression of a functional GAPDHN in *E. Coli* is not the sole variable determining growth of *E. Coli* during fermentation. Further insight into the variable conditions affecting growth of *E. Coli* even in aerobiosis are taught by Valverde at page 156, col. 2, paragraph 3, when the author states "Noteworthy, the GAPDHN-producing clone failed to grow on M63 medium supplemented with gluconeogenic substrates succinate plus acetate, showing in this medium the same pattern as the parental W3CG strain". Therefore, in aerobic or anaerobic fermentations multiple variables account for the growth of the bacterium including the carbon source.

Regarding 2) this is not dispute. However, in preferred embodiments Valverde et al., teaches that the **GAPDHN** is able to function in its natural environments which can compete with advantage over the cytosolic NAD-dependent GAPDH due to its lower Km values for G3P and the pyridine nucleotide (p. 157, col. 1, last paragraph). Indeed, Valverde et al., discloses that GAPDHN has been found in some prokaryotic microorganisms including Streptococcus strains that lack the NADPH-generating enzymes of the oxidative pentose pathway and these strains simultaneously contain the NAD-depending phosphorylating GAPDH either to generate NADPH or ATP. Thus the art reads on a metabolically engineered *E. Coli* having both GAPDH and GAPDHN and thus the claimed first metabolic pathway of the invention (e.g., an intact GAPDH which produces NADH). Note that disclosed examples and preferred embodiments do not constitute a teaching away from a broader disclosure or nonpreferred embodiments. In re Susi, 440 F.2d 442, 169 USPQ 423 (CCPA 1971). Also note that case law states that anticipation does not require the actual creation or reduction to practice of the prior art subject matter; anticipation requires only an enabling disclosure. In re Donohue, 766 F.2d 531, 533 [226 USPQ 619] (Fed. Cir. 1985). A reference may enable one of skill in the art to make and use a compound even if the author or inventor did not actually make or reduce to practice that subject matter. Bristol-Myers, 246 F.3d at 1379; see also In re Donohue, 766 F.2d at 533.

Regarding 3), the examiner recognizes that obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either in the references themselves or in the knowledge generally available to one of ordinary skill in the art. See *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988)and *In re Jones*, 958 F.2d 347, 21

USPQ2d 1941 (Fed. Cir. 1992). In this case, Nissen discloses a metabolic engineered *S. cerevisiae* wherein reduced formation of surplus NADH and an increased consumption of ATP in biosynthesis results in decreased glycerol yield and enhanced ethanol production. So if reduced formation of surplus NADH resulting in reoxidation of surplus NADH to balance NAD+/NADH ratio redirect the flux of carbon from glycerol to ethanol, modification of the G3P → 3-PGA pathway to reduce production of NADH by GAPDH at the cost of expressing the non-phosphorylating NADP+ depending **GAPDHN** should be reasonably expected to reduce NADH surplus, reduce ATP production, and thus redirect the flux of carbon from glycerol to ethanol, as G3P is the same substrate for the three separate metabolic reactions (See Fig. 4 in Valverde) . The mere modification of redox enzymes that catalyze metabolic reactions of products of industrial value derived from the central carbon metabolism by genetically engineering *S. cerevisiae* to express a heterologous **GAPDHN** enzyme to increase formation of NADPH at the cost of NADH formation in an attempt to divert the carbon flux from glycerol to ethanol during fermentation has no patentable significance unless a new and unexpected result is produced.

Regarding 4) the Examiner agrees with Applicants that prokaryotic host *E. Coli* and eukaryotic host Yeast are different host systems for expression of heterologous genes. For example, *E. coli* expression host are, in some instances, considered to be unsafe for the production of proteins by recombinant DNA methods due to their production of unacceptable by-products such as toxins. In contrast, yeast is a suitable host organism for the high-level production of secreted as well as soluble proteins, able to perform many of the post translational modifications found on human proteins. However, both *yeast* and *E. Coli* are fermentative

microorganism routinely used in the art for expression of foreign recombinant proteins, and industrially valuable products such as polymers, antibiotics, alcohol and others. The choice of which cell is used depends on the project goal and on the prosperities of the protein/ product to be produced (Watson et al, Recombinant DNA, pp. 453-455). Clearly, redox enzymes that catalyze synthesis reactions that lead to the formation of industrial important compounds are shared by both *yeast* and *E. Coli* as fermentative microorganism. Thus investigating what glycolytic pathways may be operative in eukaryotic Yeast by investigating one particular enzyme's activity in *E. Coli* as a test system should be reasonably expected to bring insight into the commonly shared glycolytic pathways of *E. Coli* and yeast.

Conclusion

Claims 1, 11-13, 17 and 18 are not allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Maria Leavitt whose telephone number is 571-272-1085. The examiner can normally be reached on M-F.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach, Ph.D can be reached on (571) 272-0739. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1633; Central Fax No. (571) 273-8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

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/Maria Leavitt/

Maria Leavitt, PhD
Examiner, Art Unit 1633